

## Microreview

# Plant models for animal pathogenesis

B. Prithiviraj,<sup>1†</sup> T. Weir,<sup>1,2†</sup> H. P. Bais,<sup>1</sup> H. P. Schweizer<sup>2,3</sup>  
and J. M. Vivanco<sup>1,2\*</sup>

<sup>1</sup>Department of Horticulture and Landscape Architecture,

<sup>2</sup>Cell and Molecular Biology Program, Colorado State University, Fort Collins, CO 80523-1173, USA.

<sup>3</sup>Department of Microbiology, Immunology and Pathology, Colorado State University, Fort Collins, CO 80523-1619, USA.

### Summary

Several bacteria that are pathogenic to animals also infect plants. Mechanistic studies have proven that some human/animal pathogenic bacteria employ a similar subset of virulence determinants to elicit disease in animals, invertebrates and plants. Therefore, the results of plant infection studies are relevant to animal pathogenesis. This discovery has resulted in the development of convenient, cost-effective, and reliable plant infection models to study the molecular basis of infection by animal pathogens. Plant infection models provide a number of advantages in the study of animal pathogenesis. Using a plant model, mutations in animal pathogenic bacteria can easily be screened for putative virulence factors, a process which if done using existing animal infection models would be time-consuming and tedious. High-throughput screening of plants also provides the potential for unravelling the mechanisms by which plants resist animal pathogenic bacteria, and provides a means to discover novel therapeutic agents such as antibiotics and anti-infective compounds. In this review, we describe the developing technique of using plants as a model system to study *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* pathogenesis, and discuss ways to use this new technology against disease warfare and other types of bioterrorism.

### Introduction

Infectious diseases transmitted by bacteria are a major cause of mortality worldwide. Contagious diseases like tuberculosis, leprosy and cholera continue to be a great threat to public health in many developing countries. Opportunistic pathogenic bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which are capable of infecting individuals who are affected by AIDS, extensive burn injury, cystic fibrosis, or are otherwise immunocompromised, have become a major concern in developed countries. Treating infections caused by such nosocomial pathogens is complicated by the emergence of drug-resistant strains and specialized physiological adaptations like biofilm formation (Costerton *et al.*, 1999). For more than half a century, a handful of antibiotics that interfere with specific metabolic events in the bacterium have been used to treat bacterial infections. However, the selective pressure mounted by this approach, the ability of bacteria to transfer plasmid-mediated genetic information and the widespread occurrence of efflux pumps contributing to intrinsic and acquired resistance (Li and Nikaido, 2004) has resulted in untreatable antibiotic resistant strains. Thus, in this post-antibiotic era we are left with a reduced capacity to fight infectious diseases, creating an urgent need to develop novel strategies for controlling bacterial infections (Hentzer and Givskov, 2003). Furthermore, the potential threat from bio-terrorism agents makes the search for novel therapeutics for infectious diseases a critically compelling area of research.

Bacteria elicit disease by deploying an array of virulence determinants that assist in their attack against host cells, leading to invasive and toxinogenic infections (Ran *et al.*, 2003). Identifying these virulence factors and determining their mechanisms of regulation and action is pivotal in designing new methods to control bacterial diseases. Traditionally, virulence factors of animal pathogenic bacteria have been discovered using costly and time-consuming biochemical and genetic approaches in animal models. More recently, methods such as *in vivo* expression technology (IVET) (Mahan *et al.*, 1993; Wang *et al.*, 1996), signature-tagged mutagenesis (STM) (Potvin *et al.*, 2003) and transposon site hybridization (TraSH) (Sasseti *et al.*, 2001), have provided more effi-

Received 4 October, 2004; revised 27 October, 2004; accepted 1 November, 2004. \*For correspondence. E-mail j.vivanco@colostate.edu; Tel. (+1) 970 491 7170; Fax (+1) 970 491 7745. †These authors contributed equally to this work.

cient, high-throughput screening abilities for the detection of bacterial virulence factors. However, the mutants developed using these techniques still need to be tested on a large number of animals and therefore are cost- and labour-intensive. In contrast, the identification of plant pathogenic virulence determinants is accomplished by fast and inexpensive screening to determine the pathogenicity of random mutants on individual plants (Willis *et al.*, 1990; Rahme *et al.*, 1991). This approach is advantageous as the gene product(s) involved in pathogenicity can readily be determined. Applying this approach to human pathogens would provide a fast and efficient model to identify and study virulence factors, and may lead to the discovery of new drug targets.

Observations that a number of Gram-negative bacteria like *P. aeruginosa*, *Burkholderia cepacia* (formerly *Pseudomonas*) and *Erwinia* sp. can infect both plants and animals (Elrod and Braun, 1942; Burkholder, 1950; Starr and Chatterjee, 1972; Fick, 1993), and that type III secretion systems, which participate in the export of virulence factors, were conserved between plant and animal pathogens (Van Gijsegem *et al.*, 1993; Hardt and Galan, 1997; Galan and Collmer, 1999; Staskawicz *et al.*, 2001), led to the development of the first plant model to study human pathogenesis. This model was developed using the plant *Arabidopsis thaliana* in conjunction with *P. aeruginosa*, and provided the first evidence that virulence factors are conserved in both plant and animal pathogenicity (Rahme *et al.*, 1995). More recent studies have demonstrated that in addition to Gram-negative human pathogens, several Gram-positive human pathogens can also infect plants (Jha *et al.*, 2005; B. Prithiviraj, H.P. Bais, A.K. Jha and J.M. Vivanco, submitted). These findings increase the usefulness of plant/human pathogen models by broadening their potential application and increasing the number of infectious diseases for which we can seek to develop novel treatments. The plant/human pathogen model is also of evolutionary interest as it bridges the divide between plant and animal pathogenesis. In this review, we will discuss plant models that have been developed to study *P. aeruginosa*, *Enterococcus faecalis* and *Staphylococcus aureus* infections and outline the insights gained from using these models to study human pathogenesis.

### ***Pseudomonas aeruginosa***

*Pseudomonas aeruginosa* (Family: Pseudomonadaceae) is an aerobic, Gram-negative, rod-shaped bacterium that inhabits soil, water and biotic surfaces. Although it is a saprophyte and rarely infects healthy tissue, it is the embodiment of an opportunistic pathogen in humans and animals. *P. aeruginosa* is primarily a nosocomial pathogen that is most commonly found in cystic fibrosis patients, burn victims and other immuno-compromised patients, as

well as soft contact lens wearers and those who use prostheses (Fick, 1993). Eradication of *P. aeruginosa* infections has remained a clinical challenge, as the bacteria are notoriously resistant to antibiotic treatment. One of the contributing factors to its antibiotic resistance is that as a soil-dwelling bacteria, *P. aeruginosa* is frequently in contact with bacilli, actinomycetes and molds, coevolving resistance to their naturally produced antibiotics (Rahme *et al.*, 1995). The main mechanism responsible for acquired and intrinsic drug resistance is synergy between an impermeable outer membrane and active efflux from the cell (Nikaido, 2003; Li and Nikaido, 2004). In addition, *P. aeruginosa* maintains transferable antibiotic resistant plasmids (Stewart, 2002) and often colonizes surfaces in its more impervious biofilm form (Singh *et al.*, 2000). Biofilm bacteria are notoriously antibiotic resistant and the molecular basis for this observation is slowly being unravelled (Costerton *et al.*, 1999; Drenkard and Ausubel, 2002; Singh *et al.*, 2002; Stewart, 2002; Mah *et al.*, 2003). In human pathogenic infections *P. aeruginosa* has proven to be recalcitrant, as it is resistant to high doses of antibiotics such as ciprofloxacin (Poole, 2000). To date, although chronic *P. aeruginosa* infections in the lungs of cystic fibrosis (CF) patients can be temporarily controlled by antibiotic treatment, in the long run they are incurable.

### ***Pseudomonas aeruginosa* – plant infection models**

Aside from being an effective and deadly opportunistic human pathogen, *P. aeruginosa* has been known to infect a number of plants (Elrod and Braun, 1942; Burkholder, 1950). However, it was Dr Rahme and coworkers, using the clinical isolate of *P. aeruginosa* strain UCBPP-PA14 and the model plant *A. thaliana* (Thalle cress), as well as *Lettuca sativa* (lettuce), who first showed that this bacterium employs a similar subset of virulence factors to elicit disease in animals and plants (Rahme *et al.*, 1995; 1997). They took advantage of the fact that in addition to producing visual disease symptoms on plant leaf surfaces, similar to plant pathogenic bacteria, *P. aeruginosa* was capable of multiplying rapidly in the apoplast, correlating with disease severity. The virulence factors *toxA* and phospholipase (*plcS*) are essential for animal pathogenesis. Inoculation with *toxA* and *plcS* mutants resulted in reduced disease severity in plant and mouse models, correlating with low bacterial load in infected tissues. Conversely, Rahme *et al.* (1995) showed that mutations in *gacA*, a virulence factor involved in pathogenesis of the plant pathogens *Pseudomonas syringae*, *P. viridiflava* and *P. marginalis*, also caused reduced virulence in a mouse model. These findings established the validity of using a plant model to study pathogenesis of animal pathogens and led to the speculation that a plant model could aid in

identification of novel virulence factors through screening of random *P. aeruginosa* mutants. To test this hypothesis, 2500 random transposon mutants of *P. aeruginosa* were inoculated on lettuce leaves and the disease severity was scored (Rahme *et al.*, 1997). Nine mutants showed reduced virulence in the plant model. Astonishingly, eight of the nine mutants that displayed attenuated virulence in the plant assay were also less pathogenic in animal infection assays. Molecular analysis of the mutants revealed that two of the mutated genes produced the known virulence factors *gacA* and *dsbA* (Peeke and Taylor, 1992; Shevchik *et al.*, 1995; Watarai *et al.*, 1995). However, seven of the mutations were in genes that showed no homology to genes known to be implicated in pathogenesis. Several of these genes appear to encode global virulence factors. For instance, insertion in the gene *mvfR* (LysR-like transcriptional regulator) showed 50% reduced haemolytic activity and no pyocyanin production, while *dsbA* mutants exhibited 60–70% reduced elastase activity and a mutation in *gacA* had significantly reduced pyocyanin production. Therefore, these loci might be involved in or coordinate the expression of other virulence factors responsible for infection of animals. As the screening of such a large number of mutants is not practical using vertebrates, the plant model provided an efficient, high-throughput screening tool for the identification of these virulence factors.

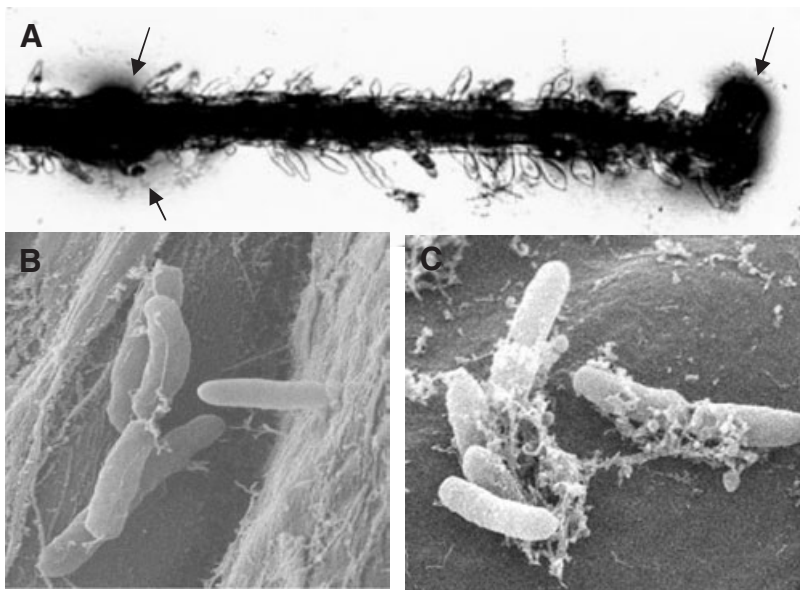
Plant models have also assisted in the characterization of several other suspected virulence factors in *P. aeruginosa*. The *degP* gene product in *E. coli* is reportedly responsible for the degradation of damaged proteins (Strauch and Beckwith, 1988), growth at high temperatures (Strauch *et al.*, 1989) and protection from heat stress (Speiss *et al.*, 1999). Homologues of *degP* have also been shown to play a role in pathogenesis in both animal (Johnson *et al.*, 1991) and plant pathogens (Stevens, 1998). The role of *mucD*, a PA14 homologue of *degP*, was elucidated using multihost pathogenesis models, including Arabidopsis as a host plant (Yorgey *et al.*, 2001). *P. aeruginosa mucD* mutants form colonies with a mucoid phenotype, which has been attributed to the overproduction of alginate (Boucher *et al.*, 1996), a virulence factor associated with chronic infection in cystic fibrosis patients (Govan and Deretic, 1996). Interestingly, a *mucD* mutant, producing more alginate, showed decreased pathogenicity in Arabidopsis, *Caenorhabditis elegans* and mouse models (Yorgey *et al.*, 2001). To clarify the role of alginate in PA14 pathogenicity, *algD*, a gene involved in alginate biosynthesis, was also mutated. The PA14 *algD* mutant did not show reduced virulence in any of the models tested. This suggests that alginate does not play a significant role as a virulence factor in Arabidopsis, *C. elegans* and mouse pathogenicity models, and may only be an important virulence factor in chronic infections

requiring biofilm formation, such as infections found in the lungs of cystic fibrosis patients (Yorgey *et al.*, 2001).

A similar multihost study was used to characterize the *rpoN* gene, which encodes an alternate sigma factor in *P. aeruginosa*. This sigma factor is implicated in virulence factor regulation in both plant and animal pathogens (Toten *et al.*, 1990; Goldberg and Dahnke, 1992), and was therefore thought to be required for multihost pathogenesis. However, an *rpoN* insertion mutation elicited disease symptoms similar to wild type in Arabidopsis by 7 days post infection, and only showed significantly less killing in the *C. elegans* model, surprisingly suggesting that *rpoN* does not regulate the expression of any genes that are universally required for virulence (Hendrickson *et al.*, 2001).

Another interesting application of the plant model is the characterization of virulence factors found in the non-typeable, less virulent strains of *P. aeruginosa* often found in chronic lung infections of cystic fibrosis patients. One of the major constraints in studying the pathogenesis of these isolates is that they usually display a dramatically attenuated virulence in classic animal infection models. For example, the *P. aeruginosa* cystic fibrosis strain FRD1 which is characteristically mucoid was tested for pathogenicity by transtracheal installation of an agar bead containing  $10^4$  bacteria in rats (Cash *et al.*, 1979; Woods *et al.*, 1991). FRD1 was unable to establish infection, and the bacterial count reduced over a period of time with only  $4.1 \times 10^1$  cfu detected in the lung homogenates 14 days post inoculation. However, the development of a wounded alfalfa (*Medicago sativa*) seedling model was sufficiently sensitive to show disease symptoms using low infectious doses of strain FRD1 (Silo-Suh *et al.*, 2002). Different virulence factor mutations were tested using this model to determine which of these factors play a role in chronic rather than acute *P. aeruginosa* infections. *RhlR* and *algT* were found to be important for virulence using the alfalfa seedling model, although mutations of other common virulence factors such as *RpoS*, *PvdS* and *LasR* did not decrease the virulence of FRD1 on alfalfa. Interestingly, the FRD1 *algD* mutant, also defective in alginate biosynthesis, did not show reduced virulence in the alfalfa seedling model, agreeing with the previously discussed conclusions of Yorgey *et al.* (2001) that suggest that alginate is not an important virulence factor in plant, *C. elegans* and mouse models. Furthermore, an *algT* mutant in a PAO1 background did not show attenuated virulence, suggesting that *algT* controls an unidentified virulence determinant that is important in an attenuated strain like FRD1, but masked in highly virulent strains of the pathogen like PAO1 (Silo-Suh *et al.*, 2002).

The mode of *P. aeruginosa* infections has been extensively studied in leaf tissue (Plotnikova *et al.*, 2000) and wounded stems (Silo-Suh *et al.*, 2002). However, these



**Fig. 1.** *Pseudomonas aeruginosa* PAO1 infects *Arabidopsis thaliana* roots.

A. *P. aeruginosa* PAO1 forms confluent biofilms on the surface of *A. thaliana* roots (arrows).

B. Colonization of *Arabidopsis* roots by *P. aeruginosa* PAO1.

C. *P. aeruginosa* cell embedded in a polysaccharide matrix.

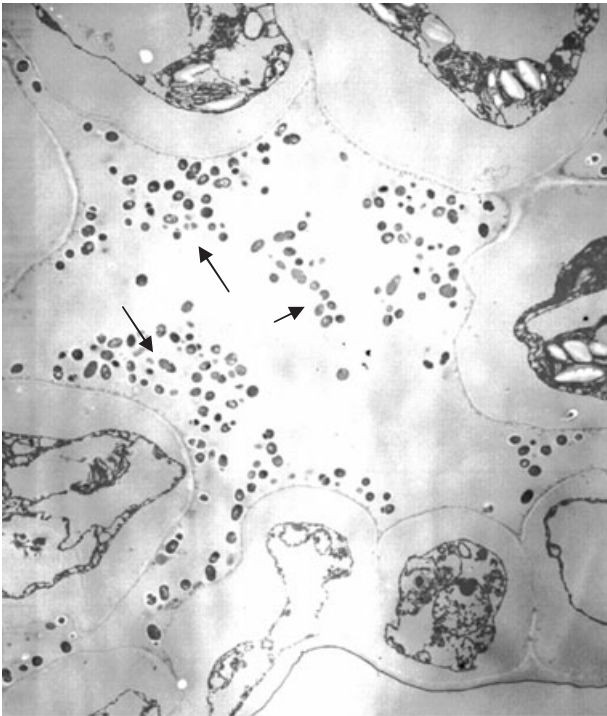
models cannot be used to study a characteristic feature of infection, biofilm formation. Recent studies have led to the development of a root infection model (Fig. 1A-C) where the biofilm formation on the root surface can be readily monitored microscopically using appropriate staining procedures (Walker *et al.*, 2004). This system has two obvious advantages: first, it provides a model for identifying compounds that limit biofilm formation and provides a platform for observing biofilm-deficient mutants. Second, screening plant roots for their susceptibility to bacterial biofilms could lead to discovery of plant-derived factors that inhibit biofilm formation and could aid in treating *P. aeruginosa* infections. Using this system, Walker *et al.* (2004) tested PAO1, the quorum-sensing mutants *lasI* and *rhII* and the *lasIrhII* double mutant for pathogenicity against *Ocimum basilicum* (basil). They found that the plant responded to bacterial root infection by increasing the secretion of the antimicrobial compound rosmarinic acid (RA) from its roots. This compound was able to kill planktonic cells, but had little effect on *P. aeruginosa* biofilms. Further, only the *lasI* mutant, but not the rhamnolipid-deficient *rhII* or *lasIrhII* mutants, retained virulence against *O. basilicum*, strengthening the hypothesis that it is the biofilm formation that confers resistance to the pathogen against the antimicrobial effect of rosmarinic acid. Interestingly, Mathesius *et al.* (2003) reported that acylated homoserine lactones (AHLs) induced changes in the secretion of plant secondary metabolites that could affect a plant's interaction with bacteria. *Medicago truncatula* was found to secrete AHL signal mimics that have the potential to interfere with quorum-sensing signalling in bacteria (Teplitski *et al.*, 2000; Bauer and Robinson, 2002). While research on the relationship between bacte-

rial quorum-sensing and plant metabolites is still a largely unexplored area, it suggests that plants may be a valuable source of novel compounds which inhibit or interfere with bacterial quorum-sensing, and which could be developed as novel anti-infective therapeutic agents.

### ***Enterococcus faecalis***

*Enterococcus faecalis* (Family: Micrococcaceae), a Gram-positive bacterium normally growing as a commensal organism in the gut, is a leading cause of nosocomial infections (Moellering, 1991; 1992). Although the ability of *E. faecalis* to cause serious disease is well recognized, not much is known about enterococcal virulence factors that contribute to its pathogenesis. Recent advances in this field have resulted in elucidation of some of the virulence factors from *E. faecalis*, including cytolysin (Cyl), a factor called aggregation substance (AS), a zinc metalloprotease (gelatinase), and *fsr* (an *E. faecalis* regulator), a putative quorum-sensing system thought to be involved in gelatinase and/or serine protease regulation (Singh *et al.*, 1998; Qin *et al.*, 2000; 2001). The limited knowledge of enterococcal virulence factors is due to the cumbersome and expensive nature of mammalian models for enterococcal infections. Recently, a *C. elegans* model, which can potentially aid in the search for new virulence factors, has been developed to study *E. faecalis* pathogenesis (Garsin *et al.*, 2001; Sifri *et al.*, 2002). Using plant infection models would further aid in our quest to understand *E. faecalis* pathogenesis.

It has recently been demonstrated that *E. faecalis* can colonize and inflict disease on the model plant *A. thaliana* (Jha *et al.*, 2005). When inoculated to leaves or roots,



**Fig. 2.** *Enterococcus faecalis* OG1RF multiplies in the intercellular spaces of *Arabidopsis thaliana* (Col-0) leaves.

three clinical isolates of *E. faecalis*, FA-2-2, V583 and OG1RF, exhibited potent pathogenicity in *A. thaliana* involving a sequential array of events, including attachment to the root surface, congregation of bacteria in stomata, colonization in intercellular spaces (Fig. 2), and formation of communities on the root surfaces (Jha *et al.*, 2005). However, the strains differ in their virulence. Interestingly, some of the *E. faecalis* virulence factors required for mammalian pathogenesis are also involved in plant pathogenicity, further indicating the validity of using plant models to study *E. faecalis* pathogenesis.

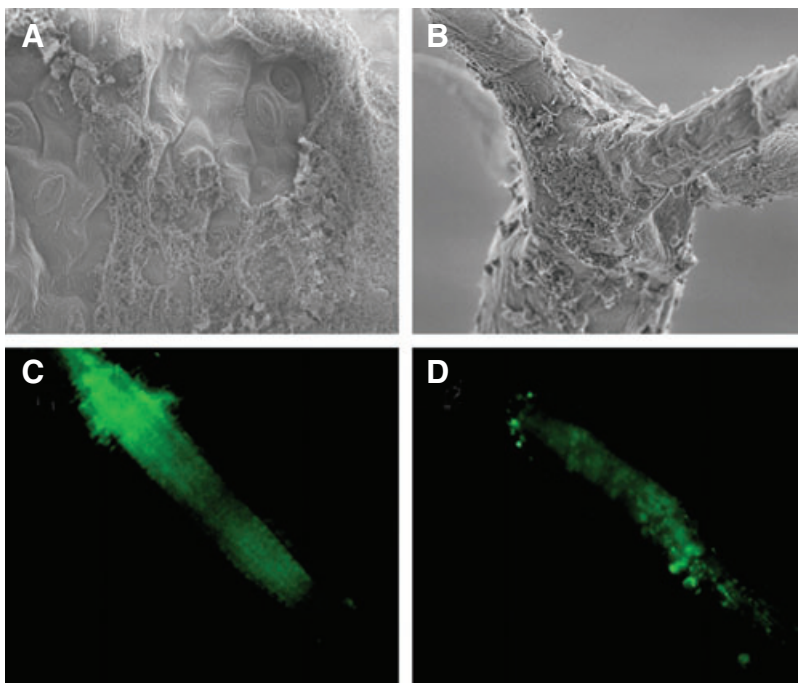
As previously outlined, quorum sensing is an important mechanism used by many prokaryotes to adapt to different environments encountered during pathogenesis (Haas *et al.*, 2002). In *E. faecalis*, the *fsr* system positively regulates the expression of the pathogenesis factors gelatinase and serine protease in a cell-density-dependent manner (Jarraud *et al.*, 2002). Qin *et al.* (2000; 2001) have characterized three genes in the *fsr* regulatory locus: *fsrA*, *fsrB* and *fsrC* using a non-polar deletion mutant in *fsrB*. They showed that *fsrB* is required for the regulatory function of the *fsr* system (Qin *et al.*, 2000; 2001). In line with the existing knowledge of the positive role of *fsr* regulatory systems in mammalian pathogenesis, Jha *et al.* (2005) found that *fsrB* plays an important role in regulating bacterial colonization of *A. thaliana* root surfaces. Accordingly, a deletion mutant,  $\Delta fsrB$ , failed to

colonize *A. thaliana* roots and exhibited attenuated pathogenicity on *Arabidopsis* plants. Along with the *fsr* system, serine protease (*sprE*) is an additional virulence factor thought to play a role in systemic disease in mammalian hosts (Qin *et al.*, 2001). The serine protease gene *sprE*, which lies immediately downstream of and is co-transcribed with *gelE*, encodes a secreted 26 kDa serine protease that shares homology with the *S. aureus* V8 protease (Qin *et al.*, 2000; 2001). Insertion disruption of *sprE* also attenuates virulence in the mouse peritonitis and *C. elegans* model systems (Sifri *et al.*, 2002). As observed in mammalian and invertebrate models (Garsin *et al.*, 2001), the serine protease deletion mutant  $\Delta sprE$  showed reduced pathogenicity in the *A. thaliana* root pathogenicity model (Jha *et al.*, 2005). The prevalence of antibiotic resistance in *E. faecalis* has reached disturbing proportions and there is a need to develop alternate antimicrobial agents to cure infections. Such an endeavour requires a better understanding of the virulence factors of *E. faecalis*. Plant infection models can aid in high-throughput screening of *E. faecalis* mutants for putative virulence factors. Furthermore, the screening of different plant species for differential infection by *E. faecalis* may provide leads to novel antimicrobial agents.

### ***Staphylococcus aureus***

*Staphylococcus aureus* (Family: Micrococcaceae) is a Gram-positive bacterium that is also capable of infecting humans and animals. In humans it causes a wide array of diseases from superficial skin infections to life-threatening systemic diseases like septicemia, pneumonia, bone infection, meningitis and endocarditis (Bradley, 2002). Several virulence factors make *S. aureus* a versatile pathogen, including cell-associated products, secreted exotoxins and regulatory proteins (Archer, 1998). The secreted exotoxins include haemolysins, nucleases, proteases, lipases, hyaluronidases and collagenases (Dinges *et al.*, 2000). In humans, these toxins have direct cytotoxic effects, impairing the immune system. The production of these toxins is controlled by regulatory genes like *sarA* and *agr* (Cheung and Zhang, 2002). Staphylococci also form biofilms, resulting in serious infections in which several other factors orchestrate to cause full virulence (Kupferwasser *et al.*, 2003).

The whole genome of *S. aureus* has been sequenced (Iandolo *et al.*, 2002). To make better use of this information it is imperative to screen mutants of this bacterium for their potential to cause infection. Therefore, it is necessary to develop simple model systems to study *S. aureus* pathogenesis. Recently, the model nematode, *C. elegans*, has been used to study the pathogenesis of *S. aureus* (Sifri *et al.*, 2003). A plant model has also been developed to test *S. aureus* pathogenicity. Leaf or root inoculation of



**Fig. 3.** *Staphylococcus aureus* NCTC 8325 infects *Arabidopsis thaliana*.

A. Extensive colonization of the leaf by *S. aureus*; note the polysaccharide matrix.

B. Colonization of the leaf trichomes.

C. Biofilm formation on the roots of wild-type *A. thaliana* (Col-0) plants.

D. *S. aureus* did not form good biofilm on the roots of transgenic *A. thaliana* (*lox2*) which accumulates high levels of salicylic acid.

*A. thaliana* with wild-type *S. aureus* elicited symptoms reminiscent of typical bacterial plant pathogens including colonization of the leaf and trichomes (Fig. 3A and B), formation of water soaked lesions, chlorosis and necrosis (B. Prithiviraj, H.P. Bais, A.K. Jha and J.M. Vivanco, submitted). However, the severity of the symptoms depended on the bacterial strains tested. Such differences in the pathogenicity of plant, animal and human pathogens are known and are thought to be due to the differences in virulence as result of differential gene expression (Choi *et al.*, 2002). Similar to the studies with mutants of *P. aeruginosa*, which suggested conservation of some of the virulence factors and regulatory factors in plant and animal pathogenesis (Rahme *et al.*, 1997), the function of virulence factors and two global transcription regulators was found to be essential for *S. aureus* pathogenesis in both animals and *A. thaliana*. Similar attenuation of virulence was observed in a *C. elegans* infection model (Sifri *et al.*, 2003). Three mutants attenuated in global regulatory systems that affect the synthesis of virulence factors, RN6911 (*agr*<sup>-</sup>), ALC 488 (*sarA*<sup>-</sup>) and ALC 842 (*agr/sar*<sup>-</sup>), showed reduced virulence on animal models (Cheung and Ying, 1994; Booth *et al.*, 1997; Chan and Foster, 1998; Blevins *et al.*, 1999; 2002; Kielian *et al.*, 2001) and were also defective in pathogenicity on *Arabidopsis thaliana*. Similarly, a mutant DU1019 (*hla*<sup>-</sup>) defective in alpha-toxin and biofilm formation (DU1019) (Beenken *et al.*, 2003) was less pathogenic in *A. thaliana*. Interestingly, *S. aureus* colonization was also influenced by host factors. Some genotypes of *A. thaliana* accumu-

late high concentrations of the phenolic compound salicylic acid (a precursor of aspirin), while others produce less (Shah, 2003). The differences in salicylic acid (SA) levels in plants have been correlated with disease resistance by a process in which SA is able to activate gene cascades involved in plant defence response against pathogens (Shah, 2003). In our studies, *S. aureus* colonization was inhibited on roots of plants that hyper-accumulated salicylic acid (Fig. 3B and C). In such roots, the bacteria were not able to form biofilms, and the lack of biofilms was correlated to a lack of virulence. Similar effects of salicylic acid on *S. aureus* was observed in animal pathogenesis models in which SA-treated bacteria showed less virulence in a *C. elegans* model. Accordingly, treatment of an invasive experimental *S. aureus* infection (endocarditis) with intravenous aspirin resulted in a significant reduction in bacterial densities within target tissues and kidneys; and SA mediated these effects (Kupferwasser *et al.*, 1999; 2003). The molecular targets of SA in decreasing *S. aureus* virulence have not been identified. These results highlight the value of using plant systems not only for the screening of virulence factors needed by bacterial pathogens to cause infection but most importantly for the discovery of novel therapeutics.

#### Plant infection models and biosecurity select agents

The increased risk of bioterrorism has become a concern in recent years. In response to increased bioterrorism awareness, the Centers for Disease Control and Preven-

tion as part of the Department of Health and Human Services (HHS) established the select agents program (<http://www.cdc.gov/od/sap>) to regulate the possession of biological agents and toxins that have the potential to pose a severe threat to public health and safety. Realizing the shared potential threat that a number of the biological agents pose to both humans and animals, a list detailing the potential overlap of high consequence HHS/USDA livestock agents was generated, which to date only includes animal pathogens. However, in light of the fact that many animal pathogens can also infect plants and cause disease symptoms similar to those in animals, some of the select agents on the overlap list, especially saprophytic bacteria like *Burkholderia* (formerly *Pseudomonas*) *pseudomallei* the causative agent of melioidosis (White, 2003), should be evaluated in plant models. The outcomes of such studies may not only be new disease models for these emerging pathogens and potential plant-derived therapeutics, but also may necessitate a evaluation of the notion that overlap agents may pose threats beyond the animal kingdom. Furthermore, a developing and complementary research area suggests that plant systems could be used as environmental indicators of the presence of such select agents, providing an early warning system of intentional releases of these agents.

## Conclusions

Using plants as model systems has proven to be an effective way to study the molecular basis of pathogenesis of several animal pathogenic bacteria. The data obtained thus far suggest considerable overlap of virulence between plant and animal pathogens. An additional advantage of using plant models over invertebrate models is the occurrence of anti-infective secondary metabolites in plants that are induced by pathogen infections. Isolation of these compounds may prove useful as novel agents in the treatment of bacterial infections (Fig. 4). Availability of the complete genome sequence of a number of pathogenic bacteria and the model plant *A. thaliana* and the ease of handling plant infection models provide an opportunity to study the molecular basis of host–pathogen interaction. Furthermore, the potential to combine several host models for bacterial infection to screen for the interapplicability of organism-secreted anti-infective compounds presents a powerful new tool for developing novel therapeutics.

## Acknowledgements

The research presented in this paper was supported by the Colorado State University Agricultural Experiment Station (J.M.V.). J.M.V. is an NSF-CAREER Faculty Fellow (MCB-0093014). H.P.S. is supported by grants from NIH.

## References

- Archer, G.L. (1998) *Staphylococcus aureus*: a well-armed pathogen. *Clin Infect Dis* **26**: 1179–1181.
- Bauer, W.D., and Robinson, J.B. (2002) Disruption of bacterial quorum sensing by other organisms. *Curr Opin Biotechnol* **13**: 234–237.
- Beenken, K.E., Blevins, J.S., and Smeltzer, M.S. (2003) Mutation of *sarA* in *Staphylococcus aureus* limits biofilm formation. *Infect Immun* **71**: 4206–4211.
- Blevins, J.S., Gillaspay, A.F., Rechten, T.M., Hurlburt, B.K., and Smeltzer, M.S. (1999) The Staphylococcal accessory regulator (*sar*) represses transcription of the *Staphylococcus aureus* collagen adhesin gene (*cna*) in an agr-independent manner. *Mol Microbiol* **33**: 317–326.
- Blevins, J.S., Beenken, K.E., Elasmri, M.O., Hurlburt, B.K., and Smeltzer, M.S. (2002) Strain-dependent differences in the regulatory roles of *sarA* and *agr* in *Staphylococcus aureus*. *Infect Immun* **70**: 470–480.
- Booth, M.C., Cheung, A.L., Hatter, K.L., Jett, B.D., Callegan, M.C., and Gilmore, M.S. (1997) Staphylococcal accessory regulator (*sar*) in conjunction with *agr* contributes to *Staphylococcus aureus* virulence in endophthalmitis. *Infect Immun* **65**: 1550–1556.
- Boucher, J.C., Martinez-Salazar, J., Schurr, M.J., and Mudd, M.H., Yu, H., and Deretic, V. (1996) Two distinct loci affecting conversion of mucoidy in *Pseudomonas aeruginosa* in cystic fibrosis encode homologs of the serine protease HtrA. *J Bacteriol* **178**: 511–523.
- Bradley, S.F. (2002) *Staphylococcus aureus* infections and

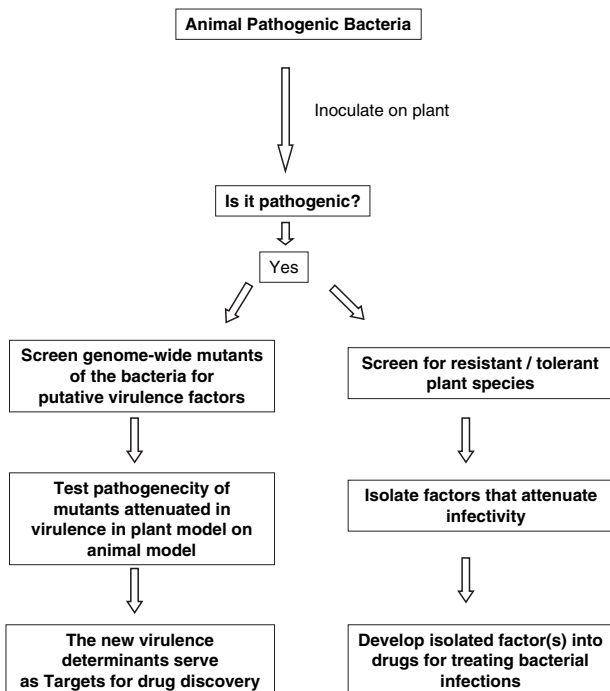


Fig. 4. Schematic representation of the advantages of developing plant models for animal pathogenic bacteria.

- antibiotic resistance in older adults. *Clin Infect Dis* **3**: 211–216.
- Burkholder, W.H. (1950) Sour skin, a bacterial rot of onion bulbs. *Phytopathology* **40**: 115–117.
- Cash, H.A., Woods, D.E., McCullough, B., Johanson, W.G., and Bass, J.A. (1979) A rat model of chronic respiratory infection with *Pseudomonas aeruginosa*. *Am Rev Respir Dis* **119**: 453–459.
- Chan, P.F., and Foster, S.J. (1998) Role of SarA in virulence determinant production and environmental signal transduction in *Staphylococcus aureus*. *J Bacteriol* **180**: 6232–6241.
- Cheung, A.L., and Ying, P. (1994) Regulation of alpha- and beta-hemolysins by the sar locus of *Staphylococcus aureus*. *J Bacteriol* **176**: 580–585.
- Cheung, A.L., and Zhang, G. (2002) Global regulation of virulence determinants in *Staphylococcus aureus* by the SarA protein family. *Front Biosci* **7**: 1825–1842.
- Choi, J.Y., Sifri, C.D., Goumnerov, B.C., Rahme, L.G., Ausubel, F.M., and Calderwood, S.B. (2002) Identification of virulence genes in a pathogenic strain of *Pseudomonas aeruginosa* by representational difference analysis. *J Bacteriol* **184**: 952–961.
- Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R., and Lappin-Scott, H.M. (1999) Microbial biofilms. *Annu Rev Microbiol* **49**: 711–745.
- Dinges, M.M., Orwin, P.M., and Schlievert, P.M. (2000) Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev* **13**: 16–34.
- Drenkard, E., and Ausubel, F.M. (2002) *Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature* **416**: 740–743.
- Elrod, R.P., and Braun, A.C. (1942) *Pseudomonas aeruginosa*; its role as a plant pathogen. *J Bacteriol* **44**: 633–645.
- Fick, R.B.J. (1993) *Pseudomonas aeruginosa* – the microbial hyena and its role in disease: an introduction. In *Pseudomonas Aeruginosa: The Opportunist – Pathogenesis and Disease*. Fick, R.B.J. (ed.). Boca Raton, Ann Arbor, London, Tokyo: CRC Press, pp. 1–5.
- Galan, J.E., and Collmer, A. (1999) Type III secretion machines: bacterial devices for protein delivery into host cells. *Science* **284**: 1322–1328.
- Garsin, D.A., Sifri, C.D., Mylonakis, E., Qin, X., Singh, K.V., Murray, B.E., et al. (2001) A simple model host for identifying Gram-positive virulence factors. *Proc Natl Acad Sci USA* **98**: 10892–10897.
- Goldberg, J.B., and Dahnke, T. (1992) *Pseudomonas aeruginosa* AlgB, which modulates the expression of alginate, is a member of the NtrC subclass of prokaryote regulators. *Mol Microbiol* **6**: 59–66.
- Govan, J.R., and Deretic, V. (1996) Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* **60**: 539–574.
- Haas, W., Shepard, B.D., and Gilmore, M.S. (2002) Two-component regulator of *Enterococcus faecalis* cytolysin responds to quorum-sensing autoinduction. *Nature* **415**: 84–87.
- Hardt, W.D., and Galan, J.E. (1997) A secreted Salmonella protein with homology to an avirulence determinant of plant pathogenic bacteria. *Proc Natl Acad Sci USA* **94**: 9887–9892.
- Hendrickson, E.L., Plotnikova, J., Mahajan-Miklos, S., Rahme, L.G., and Ausubel, F.M. (2001) Differential roles of the *Pseudomonas aeruginosa* PA14 *rpoN* gene in pathogenicity in plants, nematodes, insects, and mice. *J Bacteriol* **183**: 7126–7134.
- Hentzer, M., and Givskov, M. (2003) Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J Clin Invest* **112**: 1300–1307.
- Iandolo, J.J., Worrell, V., Groicher, K.H., Qian, Y.R., Tian, Y., Kenton, S., et al. (2002) Comparative analysis of the genomes of the temperate bacteriophages phi 11, phi 12 and phi 13 of *Staphylococcus aureus* 8325. *Gene* **289**: 109–118.
- Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., et al. (2002) Relationships between *Staphylococcus aureus* genetic background, virulence factors, agr groups (alleles), and human disease. *Infect Immun* **70**: 631–641.
- Jha, A.K., Bais, H.P., and Vivanco, J.M. (2005) *Enterococcus faecalis* uses mammalian virulence-related factors to exhibit potent pathogenicity in the *Arabidopsis thaliana* plant model. *Infect Immun* **73**: 464–475.
- Johnson, K., Charles, I., Dougan, G., Pickard, D., O'Gaora, P., Georgopoulos, C., et al. (1991) The role of a stress response protein in *Salmonella typhimurium* virulence. *Mol Microbiol* **5**: 401–407.
- Kielian, T., Cheung, A., and Hickey, W.F. (2001) Diminished virulence of an alpha-toxin mutant of *Staphylococcus aureus* in experimental brain abscesses. *Infect Immun* **69**: 6902–6911.
- Kupferwasser, L.I., Yeaman, M.R., Shapiro, S.M., Nast, C.C., Sullam, P.M., Filler, S.G., and Bayer, A.S. (1999) Acetylsalicylic acid reduces vegetation bacterial density, hematogenous bacterial dissemination, and frequency of embolic events in experimental *Staphylococcus aureus* endocarditis through antiplatelet and antibacterial effects. *Circulation* **99**: 2791–2797.
- Kupferwasser, L.I., Yeaman, M.R., Nast, C.C., Kupferwasser, D., Xiong, Y.Q., Palma, M., et al. (2003) Salicylic acid attenuates virulence in endovascular infections by targeting global regulatory pathways in *Staphylococcus aureus*. *J Clin Invest* **112**: 222–233.
- Li, X.Z., and Nikaido, H. (2004) Efflux-mediated drug resistance in bacteria. *Drugs* **64**: 159–204.
- Mah, T.F., Pitts, B., Pellock, B., Walker, G.C., Stewart, P.S., and O'Toole, G.A. (2003) A genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance. *Nature* **426**: 306–310.
- Mahan, M.J., Slauch, J.M., and Mekalanos, J.J. (1993) Selection of bacterial virulence genes that are specifically induced in host tissues. *Science* **259**: 686–688.
- Mathesius, U., Mulders, S., Gao, M., Teplitski, M., Caetano-Anolles, G., Rolfe, B.G., and Bauer, W.D. (2003) Extensive and specific responses of a eukaryote to bacterial quorum-sensing signals. *Proc Natl Acad Sci USA* **100**: 1444–1449.
- Moellering, R.C., Jr (1991) The Garrod Lecture. The enterococcus: a classic example of the impact of antimicrobial resistance on therapeutic options. *J Antimicrob Chemother* **28**: 1–12.
- Moellering, R.C., Jr (1992) Emergence of Enterococcus as a significant pathogen. *Clin Infect Dis* **14**: 1173–1176.
- Nikaido, H. (2003) Molecular basis of bacterial outer membrane permeability revisited. *Microbiol Mol Biol Rev* **67**: 593–656.
- Peeke, J.A., and Taylor, R.K. (1992) Characterization of a

- periplasmic thiol: disulfide interchange protein required for the functional maturation of secreted virulence factors of *Vibrio cholerae*. *Proc Natl Acad Sci USA* **89**: 6210–6214.
- Plotnikova, J.M., Rahme, L.G., and Ausubel, F.M. (2000) Pathogenesis of the human opportunistic pathogen *Pseudomonas aeruginosa* PA14 in *Arabidopsis*. *Plant Physiol* **124**: 1766–1774.
- Poole, K. (2000) Efflux-mediated resistance to fluoroquinolones in Gram-negative bacteria. *Antimicrob Agents Chemother* **44**: 2233–2241.
- Potvin, E., Lehoux, D.E., Kukavica-Ibrulj, I., Richard, K.L., Sanschagrin, F., Lau, G.W., and Levesque, R.C. (2003) *In vivo* functional genomics of *Pseudomonas aeruginosa* for high-throughput screening of new virulence factors and antibacterial targets. *Environ Microbiol* **5**: 1294–1308.
- Qin, X., Singh, K.V., Weinstock, G.M., and Murray, B.E. (2000) Effects of *Enterococcus faecalis* *fsr* genes on production of gelatinase and a serine protease and virulence. *Infect Immun* **68**: 2579–2586.
- Qin, X., Singh, K.V., Weinstock, G.M., and Murray, B.E. (2001) Characterization of *fsr*, a regulator controlling expression of gelatinase and serine protease in *Enterococcus faecalis* OG1RF. *J Bacteriol* **183**: 3372–3382.
- Rahme, L.G., Mindrinos, M.N., and Panopoulos, N.J. (1991) Genetic and transcriptional organization of the *hrp* cluster of *Pseudomonas syringae* pv. *Phaseolicola*. *J Bacteriol* **173**: 575–586.
- Rahme, L.G., Stevens, E.J., Wolfort, S.F., Shao, J., Tompkins, R.G., and Ausubel, F.M. (1995) Common virulence factors for bacterial pathogenicity in plants and animals. *Science* **268**: 1899–1902.
- Rahme, L.G., Tan, M.-W., Le, L., Wong, S.M., Tompkins, R.G., Calderwood, S.B., and Ausubel, F.M. (1997) Use of model plant hosts to identify *Pseudomonas aeruginosa* virulence factors. *Proc Natl Acad Sci USA* **94**: 13245–13250.
- Ran, H., Hassett, D.J., and Lau, G.W. (2003) Human targets of *Pseudomonas aeruginosa* pyocyanin. *Proc Natl Acad Sci USA* **100**: 14315–14320.
- Sasseti, C.M., Boyd, D.H., and Rubin, E.J. (2001) Comprehensive identification of conditionally essential genes in mycobacteria. *Proc Natl Acad Sci USA* **98**: 12712–12717.
- Shah, J. (2003) The salicylic acid loop in plant defense. *Curr Opin Plant Biol* **6**: 365–371.
- Shevchik, V.E., Bertoli-German, I., Robert-Baudouy, J., Robinet, S., Barras, F., and Condemine, G. (1995) Differential effect of *dsbA* and *dsbC* mutations on extracellular enzyme secretion in *Erwinia chrysanthemi*. *Mol Microbiol* **16**: 745–753.
- Sifri, C.D., Mylonakis, E., Singh, K.V., Qin, X., Garsin, D.A., Murray, B.E., et al. (2002) Virulence effect of *Enterococcus faecalis* protease genes and the quorum-sensing locus *fsr* in *Caenorhabditis elegans* and mice. *Infect Immun* **70**: 5647–5650.
- Sifri, C.D., Begun, J., Ausubel, F.M., and Calderwood, S. (2003) *Caenorhabditis elegans* as a model host for *Staphylococcus aureus* pathogenesis. *Infect Immun* **71**: 2208–2217.
- Silo-Suh, L., Suh, S.-J., Sokol, P.A., and Ohman, D.E. (2002) A simple alfalfa seedling infection model for *Pseudomonas aeruginosa* strains associated with cystic fibrosis shows AlgT ( $\sigma$ -22) and RhlR contribute to pathogenesis. *Proc Natl Acad Sci USA* **99**: 15699–15704.
- Singh, K.V., Qin, X., Weinstock, G.M., and Murray, B.E. (1998) Generation and testing of mutants of *Enterococcus faecalis* in a mouse peritonitis model. *J Infect Dis* **178**: 1416–1420.
- Singh, P.K., Schaefer, A.L., Parsek, M.R., Moninger, T.O., Welsh, M.J., and Greenberg, E.P. (2000) Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* **407**: 762–764.
- Singh, P.K., Parsek, M.R., Greenberg, E.P., and Welsh, M.J. (2002) A component of innate immunity prevents bacterial biofilm development. *Nature* **417**: 552–555.
- Speiss, C., Beil, A., and Ehrmann, M. (1999) A temperature-dependant switch from chaperone to protease in a widely conserved heat shock protein. *Cell* **97**: 339–347.
- Starr, M., and Chatterjee, A. (1972) The genus *Erwinia*: enterobacteria pathogenic to plants and animals. *Annu Rev Microbiol* **26**: 389–426.
- Staskawicz, B.J., Mudgett, M.B., Dangl, J.L., and Galan, J.E. (2001) Common and contrasting themes of plant and animal diseases. *Science* **292**: 2285–2289.
- Stevens, L. (1998) *Analysis of virulence of Pseudomonas syringae on Arabidopsis thaliana*. PhD Thesis. Department of Microbiology and Molecular Genetics, Harvard University.
- Stewart, P.S. (2002) Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol* **292**: 107–113.
- Strauch, K.L., and Beckwith, J. (1988) An *Escherichia coli* mutation preventing degradation of abnormal periplasmic proteins. *Proc Natl Acad Sci USA* **85**: 1576–1580.
- Strauch, K.L., Johnson, K., and Beckwith, J. (1989) Characterization of *degP*, a gene required for proteolysis in the cell envelope and essential for growth of *Escherichia coli* at high temperature. *J Bacteriol* **171**: 2689–2696.
- Teplitski, M., Robinson, J.B., and Bauer, W.D. (2000) Plants secrete substances that mimic bacterial N-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria. *Mol Plant Microbe Interact* **13**: 637–648.
- Totten, P.A., Lara, J.C., and Lory, S. (1990) The *rpoN* gene product of *Pseudomonas aeruginosa* is required for expression of diverse genes, including the flagellin gene. *J Bacteriol* **181**: 4790–4797.
- Van Gijsegem, F., Genin, S., and Boucher, C. (1993) Conservation of secretion pathways for pathogenicity determinants of plant and animal bacteria. *Trends Microbiol* **1**: 175–180.
- Walker, T.S., Bais, H.P., Deziel, E., Schweizer, H.P., Rahme, L.G., Fall, R., and Vivanco, J.M. (2004) *Pseudomonas aeruginosa* plant root interactions: pathogenicity, biofilm formation, and root exudation. *Plant Physiol* **134**: 320–331.
- Wang, J., Mushegian, A., Lory, S., and Jin, S. (1996) Large-scale isolation of candidate virulence genes of *Pseudomonas aeruginosa* by *in vivo* selection. *Proc Natl Acad Sci USA* **93**: 10434–10439.
- Watarai, M., Tobe, T., Yoshikawa, M., and Sasakawa, C. (1995) Disulfide oxidoreductase activity of *Shigella flexneri* is required for release of Ipa proteins and invasion of epithelial cells. *Proc Natl Acad Sci USA* **92**: 4927–4931.
- White, N.J. (2003) Melioidosis. *Lancet* **361**: 1715–1722.
- Willis, D.K., Hrabak, E.M., Rich, J.J., Barta, T.M., Lindow, S.E., and Panopoulos, N.J. (1990) Isolation and characterization of a *Pseudomonas-syringae* pathovar *syringae* mutant deficient in lesion formation on beans. *Mol Plant Microbe Interact* **3**: 149–156.

Woods, D.E., Sokol, P.A., Bryan, L.E., Storey, D.G., Mattingly, S.J., Vogel, H.J., and Ceri, H. (1991) *In vivo* regulation of virulence in *Pseudomonas aeruginosa* associated with genetic rearrangement. *J Infect Dis* **163**: 143–149.

Yorgey, P., Rahme, L.G., Tan, M.-W., and Ausubel, F.M. (2001) The roles of *mucD* and alginate in the virulence of *Pseudomonas aeruginosa* in plants, nematodes, and mice. *Mol Microbiol* **41**: 1063–1076.